Changes in growth, leaf abscission, and biomass associated with seasonal tropospheric ozone exposures of *Populus tremuloides* clones and seedlings

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Abstract: The effects of single-season tropospheric ozone (O_3) exposures on growth, leaf abscission, and biomass of trembling aspen (*Populus tremuloides* Michx.) rooted cuttings and seedlings were studied. Plants were grown in the Upper Peninsula of Michigan in open-top chambers with O_3 exposures that ranged from 7 to 92 ppm-h. Depending on the genotype, total seasonal O_3 exposure in the range of 50–92 ppm-h had negative impacts on stem, retained leaf, and root biomass accumulation and on diameter growth. Leaf abscission generally increased with increasing O_3 exposure and was the principal cause of the decrease in leaf biomass of the O_3-treated plants. Considerable genetic variation in O_3 responses occurred, as shown by differences in sensitivities among clones and among seedlings. However, the responses to O_3 of rooted cuttings and seedlings were similar when seedling means were compared with clonal means for leaf abscission, diameter growth, retained leaf biomass, and root biomass. Comparison of a single square-wave treatment (52 ppm-h) with 70 and 92 ppm-h episodic exposures suggested that the plant response to the square-wave exposure was similar to the response to the highest episodic exposure even though the 92 ppm-h episodic exposure was almost twice the square-wave exposure. Our results are consistent with previous studies that show that *P. tremuloides* is highly responsive to O_3 exposure and this response has a strong genetic component.

Résumé : Les effets sur la croissance, l'abscission des feuilles et la biomasse ont été étudiés suite à l'exposition de boutures racinées et de semis de peuplier faux-tremble (*Populus tremuloides* Michx.) à l'ozone (O_3) de la troposphère au cours d'une saison. Les plants ont été cultivés dans la partie septentrionale de la Péninsule du Michigan, aux États-Unis, dans des chambres à ciel ouvert et soumis à une exposition saisonnière à O_3 dont la concentration variait de 7 à 92 ppm-h. Selon le génotype, une exposition saisonnière à une concentration de O_3 variant de 50 à 92 ppm-h avait des effets négatifs sur la tige, l'accumulation de biomasse dans les racines et les feuilles encore présentes sur les plants ainsi que sur la croissance en diamètre. En général, l'abscission des feuilles, qui était la cause principale de la diminution de biomasse foliaire chez les plants traités avec O_3, s'intensifiait lorsque l'exposition à O_3 augmentait. Des différences de sensibilité entre les clones et les semis traduisaient une grande variabilité génétique dans les réactions à O_3. Cependant, les réactions à O_3 des boutures racinées et des semis étaient semblables lorsqu'on comparait les moyennes pour l'abscission des feuilles, la croissance en diamètre et la biomasse des racines et des feuilles encore présentes sur les plants chez les semis et les clones. La comparaison entre un traitement unique (52 ppm-h) et des expositions épisodiques à 70 et 92 ppm-h suggérait que la réponse des plants à une exposition unique était semblable à celle obtenue avec l'exposition épisodique à la plus forte concentration; même si l'exposition épisodique à 92 ppm-h soumettait les plants à une concentration presque deux fois plus élevée de

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O_3 que l'exposition unique. Nos résultats sont consistants avec les études antérieures qui montrent que *P. tremuloides* réagit fortement à une exposition à O_3 et que cette réaction comporte une forte composante génétique.

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Introduction

Tropospheric ozone (O_3) is one of the most potent and widespread phytotoxins known. This important environmental stress is estimated to cause about three billion dollars in annual losses to agricultural crops (Adams et al. 1989). One of the most significant findings of the NAPAP (National Acid Precipitation Assessment Program) Forest Response Program was that O_3 also has a significant impact on growth of forest trees across much of the United States (Barnard et al. 1991; Shriner et al. 1991). For trembling aspen (*Populus tremuloides* Michx.), O_3 is reported to cause (1) decreased growth (Wang et al. 1986; Karnosky et al. 1992) and (2) intraspecific changes in aspen populations (Berrang et al. 1986, 1989, 1991).

This paper further characterizes the growth responses of *P. tremuloides* to O_3. Specifically, it (1) examines the responses of young *P. tremuloides* plants to seasonal O_3 exposures; (2) compares the response of *P. tremuloides* clones and seedlings; and (3) contrasts the response of aspen plants with episodic and square-wave O_3 exposures.

Materials and methods

Open-top chambers and experimental treatments

Experiments were conducted in 3.1 m diameter, 2.3 m tall open-top chambers (Heagle et al. 1973) modified with frustums and rain-exclusion caps. The experiments were conducted at Michigan Technological University's Ford Forestry Center at Alberta, Mich. The episodic O_3 series contained five targeted O_3 treatments as follows: a charcoal-filtered air control (80–85% of ambient O_3 was removed), 0.5*x*, 1.0*x*, 1.5*x*, and 2.0*x*, where *x* equals a modified ambient O_3 concentration. The modified ambient profile was developed from the 1987 O_3 data supplied by the Michigan Department of Natural Resources for Washtenaw County, Michigan. This ambient profile was modified slightly so that it more closely matched the 6-year averages (1978–1983) documented by Pinkerton and Lefohn (1987). The 0.5*x*, 1.5*x*, and 2.0*x* were developed as described by Hogsett et al. (1988), using a sigmoid function. The targeted profiles for the O_3 treatments are shown in Fig. 1. Three replicate chambers were used for each of the five O_3 treatments. In addition, there were three replicate nonchambered open-air plots to determine chamber effects. The fumigation season ran from June 20 to September 16 in 1990 and from June 9 to September 14 in 1991. Total seasonal O_3 exposures ranged from 7 to 69 ppm-h in 1990 and from 22 to 92 ppm-h in 1991 (Table 1).

In 1991, another set of *P. tremuloides* plants were treated in the same open-top chambers used in 1990 with a square-wave exposure consisting of 0.1 ppm O_3 for 6 h/day (09:00–15:00, eastern standard time) for 4 days per week (Monday through Thursday for 12 weeks from June 17). The total exposure, including the amount of O_3 passing through the charcoal-filtered system during nonfumigation time, was 52.0 ppm-h. Two replicate square-wave chambers were used.

Ozone generating and monitoring equipment

Ozone was generated by an OREC model V10-0[2] O_3 generator from bottled oxygen and delivered by Teflon tubing to the charcoal-filtered air stream of the chambers. Ozone concentrations in the O_3-added chambers were monitored, using a time-shared Scanivalve system, and three TECO model 49 O_3 analyzers. Each chamber was sequentially monitored for 2 min with two or three readings per hour. Adjustments of the O_3 concentrations to match the programmed profiles were made by mass-flow controllers regulated by microcomputer. This system also served as our data acquisition system with strip-chart recordings as backup. The O_3 monitors were calibrated at the beginning of each growing season against a primary EPA standard, and they were compared daily to a Monitor Labs model 8500 O_3 calibrator. Single-point and zero checks were made daily and a multipoint calibration was made weekly. Square-wave treatments were applied manually through needle valves. Ozone concentrations in the square-wave chambers were monitored as above with values stored in a data logger.

Plant material

Rooted cuttings of *P. tremuloides* were used in both the 1990 and 1991 studies. Softwood cuttings, taken from greenhouse-grown stock plants during March 1990 and April 1991 were dipped in Rootone F and rooted in 40 × 20 × 8 cm plastic trays in a perlite–peat (1:1) mix under a foglike mist from a humidifier placed inside a plastic enclosure. Rooting generally occurred within 4 weeks, at which time the rooted plants were then removed from the mist tent and placed on a greenhouse bench. Following hardening, plants were transplanted into 37.5 cm deep × 15 cm plastic pots in a media of peat–perlite–topsoil (1:1:1), supplemented with 8 g of Sierra Osmocote (17:6:12 (N–P_2O_5–K_2O) formulation), plus micronutrients (4-month formula). In 1990, plants were grown in the greenhouse for approximately 2 months. In 1991, plants were grown in the greenhouse for approximately 1 month. Approximately 2 weeks before the start of the fumigation period, the rooted plants were transferred to the field site and placed under 50% shade cloth. Plants were moved into the chambers a few days before the O_3 treatments started.

For the 1990 growing season, four *P. tremuloides* clones (216, 253, 259, and 271; Table 2) were exposed to the O_3 treatments. These clones had previously been tested for foliar O_3 sensitivity (Berrang et al. 1991) and for growth

[2] Mention of trade names or commercial products does not construe endorsement or recommendation for use.

Fig. 1. Targeted ozone profiles for the month of June, July, and August. We used these as our targeted profiles in both 1990 and 1991. These profiles are based on modified ambient ($1x$) from southern Michigan. Sigmoidal weighting was used to develop the $0.5x$, $1.5x$, and $2x$.

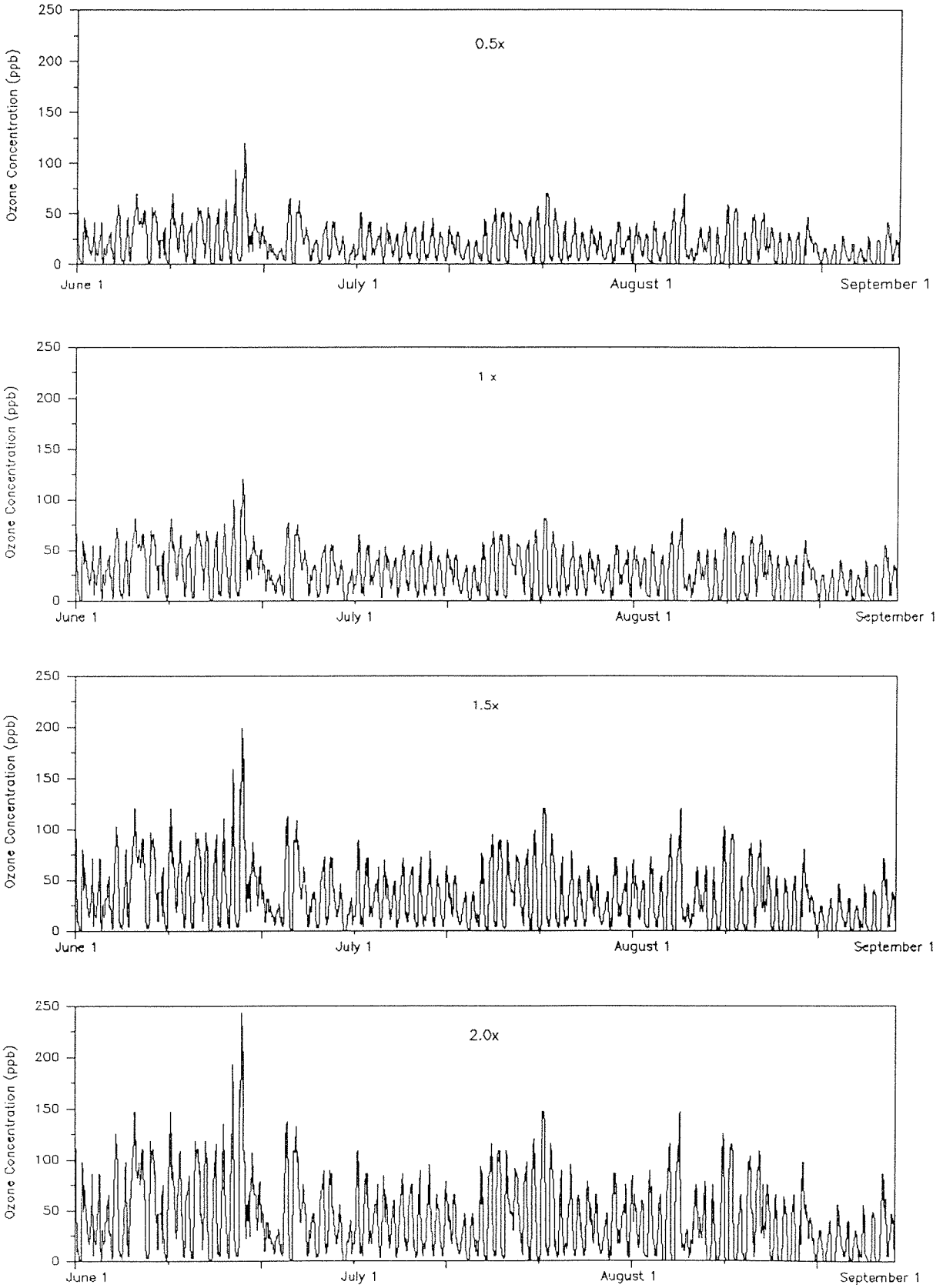


Table 1. Summary of ozone exposures for the 1990 and 1991 growing seasons.

	Treatment					
Description[†]	CF	0.5*x*	1*x*	1.5*x*	2*x*	Open-air plot
			1990			
7-h	7	32	43	52	63	30
n_{60}	0	17	102	274	402	21
n_{80}	0	0	9	80	197	0
n_{100}	0	0	4	42	79	0
n_{120}	0	0	2	12	24	0
TE	7	35	50	56	69	44
			1991			
7-h	11	40	45	61	66	39
n_{60}	0	189	234	480	669	64
n_{80}	0	58	77	159	259	0
n_{100}	0	24	38	45	84	0
n_{120}	0	13	22	27	36	0
TE	22	66	70	87	92	65

Note: CF, charcoal filtered.

[†]7-h, June–September mean of the highest 7 h of the day (ppb); n_{60}, number of hourly occurrences >0.06 ppm ozone; n_{80}, number of hourly occurrences >0.08 ppm ozone; n_{100}, number of hourly occurrences >0.10 ppm ozone; n_{120}, number of hourly occurrences >0.12 ppm ozone; TE, total seasonal exposure in ppm-h.

responses to season-long, square-wave O_3 exposures (Karnosky et al. 1992). They were selected to represent the range of responses previously found in our larger population studies. Five plants per clone were randomly assigned to the various chambers for destructive harvest at the end of the single-season growth study. Another subset of five plants per clone were randomly assigned to the various chambers for a second-year reflush study.

For the 1991 growing season, we compared responses of three clones (216, 259, and 271) with that of seedlings. For the clones, softwood cuttings were rooted in the spring of 1991 as described above. Seedlings were produced from seed obtained from flower pollinations on cut branches of five female trees with a mix of pollen from 10 male trees. Flowers were pollinated in the greenhouse in late January 1991 and seed was collected in February 1991. All parent trees were from Houghton County, Michigan, separated by at least 1 km, and chosen at random. Seed was germinated in March 1991 in plastic trays in a perlite–peat (1:1) mix. Seedlings and rooted cuttings were brought to the exposure site 1 week before the exposure season in 1991 and treated as described above. Sixteen seedlings and five plants per clone were randomly assigned to each chambered or nonchambered plot for the 1991 seedling–clone and episodic – square-wave studies. All of these plants were destructively harvested at the end of the growing season. Plants were separated into leaves, stems, and roots; oven-dried; and weighed.

Plants were watered daily with a drip irrigation system calibrated to give uniform water delivery. Plant heights, diameters, number of leaves (present and abscised), and visible foliar symptoms were measured repeatedly at the beginning of each fumigation season and at approximately 3-week intervals during the season. The average height of the rooted cuttings at the start of the fumigation season was 77.8 cm in 1990 and 24.5 cm in 1991. The average height of the seedlings in 1991 was 8.9 cm at the start of the treatment period. A random sample of 10 plants per clone and of 20 seedlings was harvested to characterize leaf, stem, and root biomass at the start of each fumigation season.

Plants from the 1990 clonal material held over winter for reflush in 1991 were maintained in a cold-storage building over the winter, placed outdoors in April 1991, and allowed to reflush under ambient air conditions. On May 15, when these plants had fully broken bud and new shoots were 10–15 cm in length with fully expanded leaves, plants were harvested and separated into old stem, new stem, leaves, fine roots, and coarse roots; oven-dried; and weighed.

Environmental variables measured

All environmental variables were recorded with a Campbell 21x data logger. Air and pot soil temperatures were measured continuously with thermocouples inside one chamber and in one open-air plot. Light intensity was monitored in all chambers and in one open-air plot, with photodiodes. Air temperature and relative humidity were monitored continuously in one chamber and in an open-air plot with air temperature and relative humidity probes. Measurements of each of the environmental variables were averaged hourly.

Statistical design and analyses

The experimental design for the chamber assignments to given treatments for all studies was a completely random design. Repeated measures of stem diameter, stem height and leaf abscission rate were analyzed with univariate and multivariate analyses of covariance (ANCOVA) for a split-plot design to test for O_3 treatment, time, and clone main effects and their interactions for each year separately. Initial stem volume calculated as the product of height and diameter squared was used as the covariate. Univariate and multivariate ANOVA were obtained for final harvest leaf, stem, and root biomass. The relative errors among plants were similar throughout the experiment so that ANOVA was justified. Whole-plot O_3 treatment effects were tested with the chamber within treatment term as the denominator in the standard variance ratio test. Clone and clone × treatment interaction terms were tested using the split-plot error term in the variance ratio test. Time and O_3 treatment orthogonal polynomial contrasts and interactions of polynomial contrasts were obtained to test for main and interaction effects and to determine the degree of polynomial fit of growth curves and exposure–response equations, respectively. Time and interaction terms involving time were tested with multivariate ANOVA and the Greenhouse–Geisser adjusted univariate ANOVA when appropriate.

The seasonal total exposure for O_3 for each chamber was used as the independent variable in the post-ANOVA regression analysis to fit O_3 exposure–response curves for each clone and time of measurement. Repeated measures of physiological and morphological variables were described

Table 2. Origin and background ozone-sensitivity information of the *Populus tremuloides* plants in this study.

Plants	Origin (county)	Foliar ozone sensitivity[†]	Growth ozone sensitivity[‡]
Clone 216	Wisconsin (Bayfield)	Tolerant	Tolerant
Clone 253	Michigan (Leelanau)	Sensitive	Sensitive
Clone 259	Indiana (Porter)	Sensitive	Sensitive
Clone 271	Indiana (Porter)	Intermediate	Intermediate
Seedlings	Michigan (Houghton)	Untested	Untested

[†]From Berrang et al. (1991).
[‡]From Karnosky et al. (1992).

Table 3. Probabilities of variance ratio tests for main and interaction effects of plant growth responses of four *Populus tremuloides* clones to 1990 ozone exposures.

Effect[†]	Height	Diameter	Leaf abscission	Retained leaf biomass	Stem biomass	Root biomass	Total
			All clones				
Tmt.	0.248	0.098	0.048	0.058	0.026	0.052	0.034
Time	0.047	0.001	0.008				
Tmt.×time	0.152	0.599	0.004				
Clone	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Tmt.×clone	0.325	0.236	0.014	0.052	0.314	0.011	0.075
Time×clone	<0.001	0.169	0.142				
Tmt.×time×clone	0.450	0.428	0.009				
			Clone 216				
Tmt.	0.005	0.492	0.010	0.087	0.138	0.122	0.140
Time	0.304	0.325	0.004				
Tmt.×time	0.135	0.384	0.044				
			Clone 253				
Tmt.	0.947	0.026	0.877	0.306	0.960	0.107	0.487
Time	0.943	0.465	0.031				
Tmt.×time	0.780	0.528	0.607				
			Clone 259				
Tmt.	0.617	0.424	0.001	0.076	0.007	0.054	0.023
Time	0.002	0.795	0.509				
Tmt.×time	0.620	0.575	0.205				
			Clone 271				
Tmt.	0.839	0.436	0.392	0.059	0.232	0.084	0.057
Time	0.566	0.639	0.301				
Tmt.×time	0.625	0.552	0.345				

Note: Values indicate significance level. Leaf, stem, and root biomass were determined at the end of the growing season and have no repeated measurements.
[†]Tmt., ozone treatment (See Table 1); time, repeated measurement during the growing season.

with polynomial models. Ozone exposure – plant response curves were obtained for individual clones and each measurement period using polynomial and the non-linear Weibull models. Data from the unchambered plots were not included in the analyses. A logarithmic transformation was made of root biomass data to stabilize the variance.

Results

Clonal responses

The episodic exposures were designed to mimic natural O_3 daily profiles. Ambient O_3 concentrations varied widely from day to day and from season to season. However,

Table 4. Probabilities of variance ratio tests for main and interaction effects for multivariate analysis of plant growth responses of three *Populus tremuloides* clones to 1991 ozone exposures.

Effect[†]	Height	Diameter	Leaf abscission	Retained leaf biomass	Stem biomass	Root biomass	Total
			All clones				
Tmt.	0.252	0.060	0.011	0.033	0.156	0.006	0.022
Time	<0.001	<0.001	0.003				
Tmt.×time	0.052	0.032	0.084				
Clone	<0.001	0.021	0.076	0.664	<0.001	0.004	0.021
Tmt.×clone	0.007	0.579	0.895	0.402	0.314	0.038	0.086
Time×clone	0.001	0.012	0.076				
Tmt.×time×clone	0.007	0.453	0.856				
			Clone 216				
Tmt.	0.523	0.175	0.118	0.169	0.282	0.038	0.107
Time	0.005	0.039	0.154				
Tmt.×time	0.369	0.238	0.272				
			Clone 259				
Tmt.	0.016	0.017	0.007	0.086	0.197	0.002	0.018
Time	0.268	0.457	0.002				
Tmt.×time	0.014	0.281	0.260				
			Clone 271				
Tmt.	0.314	0.043	0.026	0.364	0.790	0.195	0.409
Time	0.033	0.016	0.470				
Tmt.×time	0.111	0.391	0.072				

Note: Values indicate significance level. Leaf, stem, and root biomass were determined at the end of the growing season and have no repeated measurements.

[†]Tmt., ozone treatment (See Table 1); time, repeated measurement during the growing season.

because of the episodic nature of the exposures, it is difficult to provide exact targeted seasonal exposures. The 0.5*x* O_3 exposure was close to the open-air plot ambient exposure in both 1990 and 1991 (Table 1). The 0.5*x* treatment was always higher and the 2*x* was always lower than the targeted exposures when summed at the end of the season. If the charcoal-filtered treatments are excluded, total O_3 exposures ranged from 35 to 69 ppm-h and 66 to 92 ppm-h in 1990 and 1991, respectively (Table 1).

Multivariate analyses of clonal responses for the most important growth and biomass parameters showed that O_3 at seasonal exposures up to 92 ppm-h had significant effects on height, diameter, retained leaf biomass, stem biomass, and root biomass (Tables 3 and 4). Responses to the O_3 treatments were essentially the same for both the 1990 and 1991 exposure season. Differences in clonal response (e.g., stem biomass) to O_3 exposure were observed and these differences were consistent from year to year.

Initial plants were considerably larger in 1990 than in 1991 (Tables 5 and 6). This was due to the later propagation date (April) for the 1991 plants as compared with the propagation date (March) for the 1990 plants. Regardless of the year or the size of the plants, the responses to O_3 were similar in the 2 years. Compared with charcoal-filtered plants, the 1*x* treatment decreased retained leaf, stem, and root biomass (an average across all clones of 15, 16, and 13%, respectively, in 1990 and 12, 26, and 18%, respectively, in 1991). The 2*x* treatment decreased retained leaf, stem, and root biomass on average across all clones by 43, 21, and 33%, respectively, in 1990 and 27, 20, and 39%, respectively, in 1991. Leaf and root biomass from the trees in the open-air plots was comparable with plants grown under a similar exposure regime in the 1*x* chambers. Height growth and biomass of the plants in the 1*x* chambers were greater than in the open-air plots and are likely the result of a chamber effect.

Clonal variation in O_3 response was evident in both years and for all biomass components. The 2*x* treatment decreased total biomass from 8% with clone 216 to 41% with clone 259 in 1990 and from 19% with clone 271 to 45% with clone 259 in 1991. The least affected clone in each year was clone 216, and the most sensitive clone each year was clone 259. Clone 271 showed intermediate sensitivity to ozone each year.

The exposure–response curves for leaf abscission, stem biomass, and root biomass showed the effect of O_3 exposure on plant growth and were similar for both years. Leaf abscission generally increased with increasing O_3 exposure (Fig. 2*a*). Clonal differences occurred in the shape of the response curves for leaf abscission because of differences in foliar sensitivity of the clones. Stem biomass was significantly decreased by increasing O_3 exposure for

Table 5. Biomass components at the end of the 1990 growing season of four *Populus tremuloides* clones fumigated in open-top chambers with various ozone exposures.

Treatment[†]	All clones	Clone 216	Clone 253	Clone 259	Clone 271
			Retained leaf biomass (g)		
CF	15.9±1.5	10.1±1.4	16.3±1.7	18.4±2.2	18.2±1.9
0.5*x*	16.7±1.5	14.3±1.3	14.1±1.6	20.0±2.4	18.4±1.9
1.0*x*	13.8±1.5	10.8±1.3	13.9±1.7	14.3±2.1	16.2±1.9
1.5*x*	14.6±1.7	11.5±1.5	14.2±1.9	15.4±2.5	17.1±2.2
2.0*x*	8.9±1.7*	7.7±1.5	10.3±1.9	9.1±2.5*	8.8±2.2*
OP	12.5±0.6	10.8±1.3	12.0±1.4	15.1±0.6	12.5±0.6
			Stem biomass (g)		
CF	32.7±1.6	20.5±2.5	32.3±3.0	30.6±2.0	46.1±3.5
0.5*x*	33.7±1.6	29.1±2.4	29.4±2.9	30.4±2.2	44.8±3.4
1.0*x*	27.1±1.6*	22.1±2.4	26.6±3.0	20.4±1.9*	39.1±3.4
1.5*x*	28.1±1.8	20.6±2.7	28.7±3.3	23.7±2.3	39.0±3.9
2.0*x*	25.9±1.8*	21.0±2.7	30.4±3.4	18.8±2.2*	34.8±4.0
OP	23.5±1.2	22.1±2.3	22.1±2.9	21.6±1.6	27.7±2.4
			Root biomass (g)		
CF	34.2±2.2	20.7±2.6	35.1±2.2	39.7±3.2	40.1±2.7
0.5*x*	33.8±2.2	29.8±2.6	29.9±2.1	36.4±3.4	38.8±2.6
1.0*x*	30.0±2.1	23.7±2.6	29.0±2.2	30.4±3.0	36.0±2.6
1.5*x*	29.1±2.4	21.4±2.9	28.5±2.4	31.0±3.6	34.6±3.0
2.0*x*	23.7±2.4*	19.8±2.9	25.0±2.5*	23.6±3.5*	27.7±3.1*
OP	32.2±1.6	31.6±3.9	29.8±3.4	36.5±3.5	30.9±2.6
			Total biomass (g)		
CF	82.8±5.0	51.3±6.3	83.7±6.8	88.7±7.8	105.2±6.8
0.5*x*	84.9±4.9	72.2±6.1	76.0±6.6	86.7±5.2	102.1±6.6
1.0*x*	71.2±4.8	56.7±6.1	69.4±6.8	66.3±5.4	91.3±6.6
1.5*x*	72.1±5.5	53.4±6.9	72.8±7.5	69.9±7.6	90.6±7.6
2.0*x*	58.6±5.5*	48.4±6.8	66.2±7.6	51.6±7.5*	71.5±7.7*
OP	68.2±3.2	64.5±6.8	63.9±7.6	73.1±5.2	71.1±6.0

Note: Values are means ± SE. Clonal means are the average of 60 plants, 15 plants per clone and per treatment.

[†]CF, charcoal-filtered air; *x*, ambient ozone level; OP, open-air plot data (not included in statistical analyses).

*Significantly different at the 0.05 level from the charcoal-filtered air treatment, as determined by Dunnett's test.

all clones (Fig. 2*b*). Clonal differences occurred in the degree of the response. For example, the response curve of clone 259 had the greatest negative slope, while that of clone 271 had the smallest slope. The trend towards increasingly steep decreases in root biomass as O_3 exposure increased (Fig. 2*c*) was similar for the three clones, and was best explained by a Weibull equation.

Comparisons of the total number of leaves produced by the plants when averaged for all clones showed that there were no statistically significant differences across treatments (data not shown). However, there were differences among the clones as final number of leaves averaged across all treatments varied from 42 for clone 259 to 62 for clone 271. Size of individual leaves was also not affected by O_3 exposure (data not shown). Although average leaf area per leaf decreased in all treatments through the growing season, there was no additional effect from O_3 exposure.

Foliar symptoms were visible within 2 weeks after the start of the fumigation season each year and consisted of black bifacial necrosis of irregular size and shape. The black gradually faded to gray after several weeks. Occasionally, general chlorosis and (or) upper leaf surface black stipple were observed. The number of symptomatic leaves increased in all O_3 treatments during the growing season (Table 7) and increased with increasing O_3 exposure (Fig. 3). Clonal variation in foliar symptoms was detected by early August. Clone 259, previously characterized as foliar sensitive in short-term fumigations (Berrang et al. 1991), was again the most sensitive clone.

Table 6. Biomass components at the end of the 1991 growing season of three *Populus tremuloides* clones fumigated in open-top chambers with various ozone exposures.

Treatment†	All clones	Clone 216	Clone 259	Clone 271
		Retained leaf biomass (g)		
CF	9.0±0.4	9.3±0.7	9.7±0.7	7.9±0.8
1*x*	8.2±0.4	7.7±0.7	8.1±0.7	8.8±0.8
2*x*	6.9±0.4*	7.0±0.7	6.7±0.7*	7.0±0.8
Mean	8.1±0.2	7.9±0.4	8.4±0.5	7.9±0.3
		Stem (g)		
CF	9.5±0.6	10.4±1.1	8.3±0.9	9.5±0.9
1*x*	7.6±0.6	7.7±1.1	6.5±0.8	8.6±0.9
2*x*	7.7±0.6	8.7±1.1	5.6±0.9	8.9±0.9
Mean	8.3±0.3	8.9±0.5	6.9±0.4	9.0±0.3
		Root (g)		
CF	19.8±0.9	19.8±1.3	20.5±1.2	19.2±1.8
1*x*	16.3±0.9*	15.4±1.3	15.2±1.1*	18.2±1.8
2*x*	12.1±0.9*	13.1±1.3*	9.3±1.1*	14.0±1.8
Mean	16.1±0.5	16.1±0.8	15.0±1.1	17.1±0.7
		Total biomass (g)		
CF	38.3±1.9	39.6±2.9	39.0±2.9	36.6±3.5
1*x*	32.1±1.8	30.8±2.9	29.8±2.6	35.6±3.4
2*x*	26.9±1.8*	28.6±3.0*	21.6±2.6	29.9±3.4
Mean	32.4±0.8	32.9±1.6	30.3±1.9	34.0±1.3

Note: Values are means ± SE. Clonal means are the average of 45 plants, 15 plants per clone and per treatment.

†CF, charcoal-filtered air; *x*, ambient ozone level.

*Significantly different at the 0.05 level from the charcoal-filtered air treatment, as determined by Dunnett's test.

When *P. tremuloides* clonal material was exposed to square-wave and episodic exposures of similar total exposure of 52 ppm-h, decreases in stem and root biomass were greater for the square-wave treatment, which had more hourly concentrations greater than or equal to 0.1 ppm than the 0.5*x* and 1*x* exposures. The square-wave treatment had as much impact on stem and root biomass as the 2*x* ambient episodic exposure even though the total O_3 exposure of the square wave was about one half of that of the 92 ppm-h episodic exposure (Table 8).

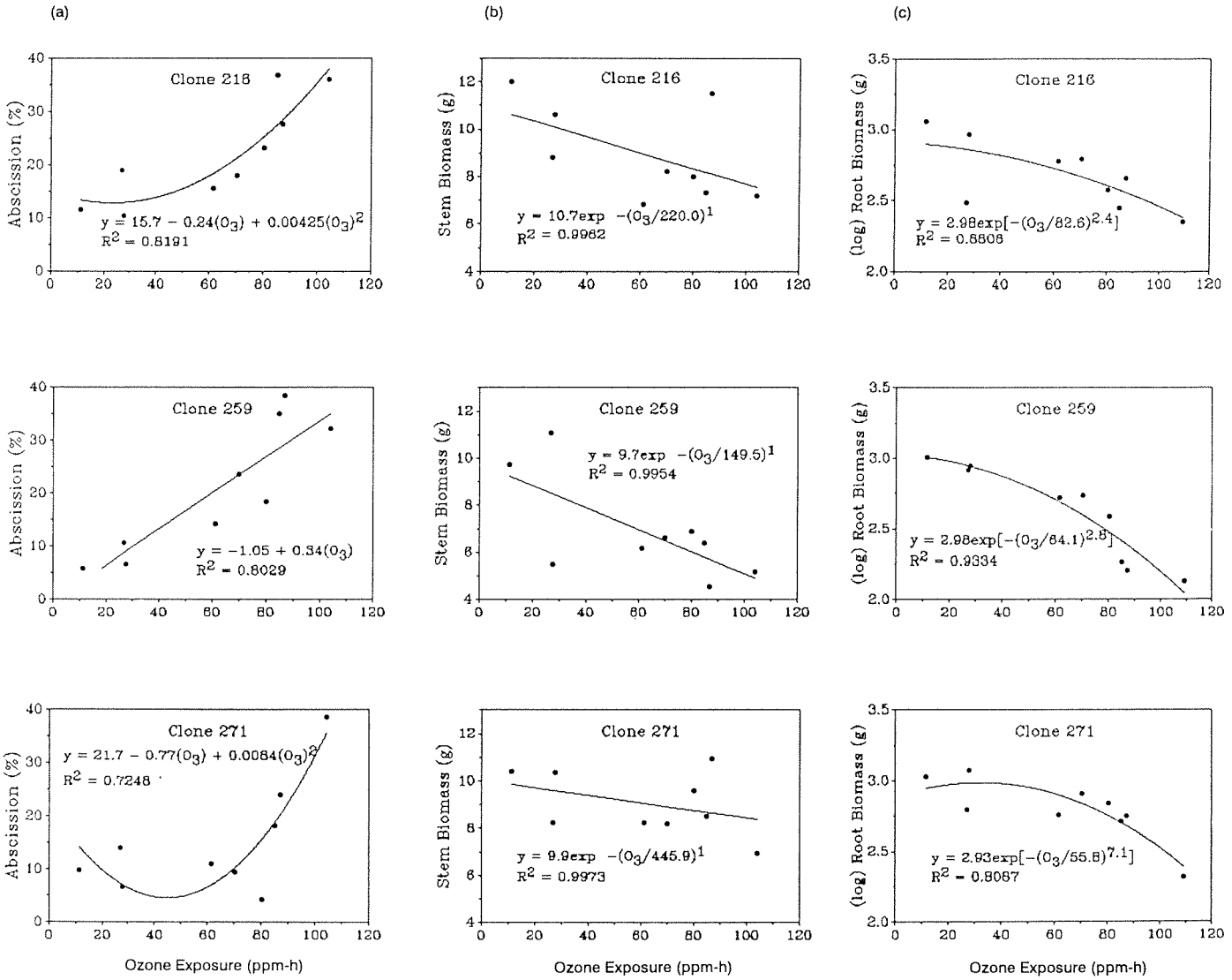
To determine the carryover effect of a previous season's O_3 exposure on the next season's initial growth, we examined the bud break and biomass of the *P. tremuloides* clones held over the winter. No statistical differences were seen for date of bud break or percentage of buds breaking (data not shown). Similarly, the amount of new stem and new leaf produced by the first flush of growth did not differ significantly among exposures (Fig. 4*a*). While we did not differentiate between new and old roots, the pattern of decreased root biomass with increasing O_3 exposures was also found in the carryover study (Fig. 4*b*).

Seedling responses

To compare the O_3 responses of our clonal material to seedlings from local *P. tremuloides* populations, we grew both clones and seedlings side by side in the open-top chambers in 1991. As with the clonal material, seedling plants grown in the open-air plots were smaller than those grown in the charcoal-filtered chambers (Table 8). Decreases in seedling biomass in response to O_3 were similar to the clonal average decrease in biomass (Table 8), but were not statistically significant at the 0.05 level because of large experimental variation (Table 9). Ozone treatments increased leaf abscission (Fig. 5) and decreased seedling growth with the 2*x* treatment; root biomass decreased 34% and total biomass decreased 26% as compared with control plants (Table 10). Ozone effects on stem height late in the growing season were small (Fig. 6*a*). Seedling exposure response curves indicated that diameter, root biomass, and stem biomass responses decrease linearly with increasing ozone exposure (Figs. 6*b*, 7*a*, and 7*b*). Stem and root biomass decreased 20% and 14%, respectively, with the 70 ppm-h O_3 exposure and 19% and 34% with the 92 ppm-h O_3 exposure, but these decreases are within the range of experimental variation.

Experimental variation in response to O_3 exposure was greater in the seedling population compared with the clones in all the measured experimental variables (Table 11). Coefficients of variation ranged from 20 to 51% for seedlings and from 12 to 36% for clones. Such variation

Fig. 2. Leaf abscission (*a*), stem biomass (*b*), and root biomass (*c*) responses to different total seasonal ozone exposures for three *Populus tremuloides* clones. Potted first-year plants were grown in open-top chambers and biomass was determined at the end of the 1991 growing season. The data points represent ozone exposures for individual chambers and are the mean of five plants per clone.



could be expected given the potential genetic variation in the seedling population. These results also show the value of using clonal material when experimental design dictates that only a small number of plants can be used, as in open-top chamber studies. Care must, of course, be taken to ensure that the clonal material is representative of the species.

Discussion

Our results are consistent with previous studies that show that *P. tremuloides* is highly responsive to O_3 exposure (Berrang et al. 1986, 1989, 1991; Karnosky 1976, 1977; Wang et al. 1986). The increased rates of leaf senescence and decreased biomass production that we observed for *P. tremuloides* are typical of those reported previously (Frost et al. 1991; Kargiolaki et al. 1991; Keller 1988; Mooi 1981; Reich and Lassoie 1985; Wang et al. 1986), except that our responses occurred with quite low O_3 exposures. Seasonal O_3 exposures of 43–92 ppm-h caused visible foliar injury, increased leaf abscission, and decreased growth and biomass accumulation. These O_3 exposures (seasonal 7-h mean concentrations of 43–66 ppb) are well within the range of reported seasonal mean O_3 concentrations for forested areas of the eastern United States (Lefohn and Jones 1986; Lefohn and Pinkerton 1988; Pinkerton and Lefohn 1987).

Number of leaves produced, leaf size, leaf weight, and height growth were not generally affected by O_3 in our study. Previously, we showed that *P. tremuloides* leaf initiation rate, mature leaf length, and internode length were insensitive to O_3 (Li et al. 1991). Since leaf initiation and internode elongation in *P. tremuloides* were not affected by O_3 in that study, it is not surprising that height growth

Table 7. Percentage of symptomatic leaves for the 1990 growing season of four *Populus tremuloides* clones fumigated in open-top chambers with various ozone exposures.

Treatment[†]	July 16, 1990	August 2, 1990	August 23, 1990
	Clone 216		
CF	9.8±2.2*c*	14.9±3.3*c*	22.9±6.9*c*
0.5*x*	15.2±4.2*b*	27.9±7.7*bc*	28.4±4.9*c*
1.0*x*	17.6±3.5*b*	43.1±6.5*b*	52.3±3.9*b*
1.5*x*	19.4±5.3*b*	44.2±7.8*b*	51.7±7.4*b*
2.0*x*	31.9±5.5*a*	65.4±5.4*a*	77.2±6.6*a*
Mean	19.0±2.3	39.2±4.1	46.5±4.4
	Clone 253		
CF	9.4±4.1*c*	9.4±3.7*c*	18.5±8.2*c*
0.5*x*	15.3±1.5*c*	25.7±6.8*b*	32.8±6.3*bc*
1.0*x*	18.0±3.9*bc*	40.9±2.9*b*	45.6±3.1*b*
1.5*x*	27.0±3.2*b*	46.0±5.5*ab*	53.1±3.0*b*
2.0*x*	38.3±4.7*a*	56.3±6.5*a*	74.2±7.2*a*
Mean	22.2±2.4	35.7±2.7	44.8±4.3
	Clone 259		
CF	20.7±2.1*a*	21.5±3.1*c*	22.6±3.4*c*
0.5*x*	21.3±1.1*a*	45.5±6.3*b*	47.4±6.6*b*
1.0*x*	19.9±3.0*a*	61.6±6.1*b*	69.2±6.6*b*
1.5*x*	27.3±2.5*a*	61.9±12.6*b*	70.3±10.4*b*
2.0*x*	34.5±11.2*a*	80.6±3.8*a*	93.8±3.6*a*
Mean	23.8±2.0	53.6±4.7	59.9±5.4
	Clone 271		
CF	7.1±1.2*b*	9.6±1.8*c*	9.5±1.7*c*
0.5*x*	6.6±1.7*b*	34.6±6.3*b*	25.9±8.7*bc*
1.0*x*	7.9±1.1*b*	54.0±5.4*a*	44.1±7.2*b*
1.5*x*	13.2±3.2*ab*	59.4±5.8*a*	46.8±7.6*b*
2.0*x*	18.2±5.0*a*	68.4±4.5*a*	77.9±3.7*a*
Mean	10.3±1.3	45.3±4.4	40.9±5.0

Note: Values are means ± SE. Clonal means are the average of 60 plants, 15 plants per clone and per treatment. Symptomatic leaves were considered to be those that either abscised prematurely or that were showing typical ozone symptoms, including black bifacial necrosis, chlorosis, or upper leaf surface stipple. Treatments with the same letter were not significantly different at the 0.05 level, as determined by the Student–Newman–Keuls multiple-comparisons test.

[†]CF, charcoal-filtered air; *x*, ambient ozone level.

in our current study was not as markedly affected as other growth parameters. Similar effects of O_3 on leaf growth were found with the hybrid poplar *Populus* ×*euramericana* (Dode) Guinier, as expanding leaves were not affected by O_3 (Frost et al. 1991). In our study, expanding leaves also showed no visible symptoms to O_3 and did not abscise. These expanding leaves import much of the carbon required for growth from recently mature leaves that allocate most of their fixed carbon upwards to the developing leaf zone (Dickson 1986). Thus, growth responses in the developing leaf zone are dependent on the recently mature leaves. We have also shown that recently mature leaves in O_3-treated *P. tremuloides* plants may compensate somewhat for the loss of mature leaves on the lower stem by exporting a greater percentage of carbon downward to the roots (Coleman et al. 1995*a*). Our results regarding leaf initiation rate and leaf growth are different from those reported by Gunthardt-Goerg et al. (1993), who reported that O_3 decreased the leaf initiation rate and the leaf size of silver birch (*Betula pendula* Roth) plants.

Root growth was generally one of our most sensitive indicators of chronic O_3 exposure, as has been reported by Cooley and Manning (1987) and Heck et al. (1986). Similar decreases in root biomass have been found for other tree species exposed to O_3 (Shafer and Heagle 1989; Qiu et al. 1992). Mature *Populus* leaves on the lower stem transport most of their carbon downward to stem and roots (Dickson 1986). Therefore, it follows that the premature abscission of these mature leaves due to O_3 stress would have a major impact on root growth.

Table 8. Comparison of the effects of square-wave and episodic ozone exposures on biomass production of three *Populus tremuloides* clones fumigated in open-top chambers with various ozone exposures in the 1991 growing season.

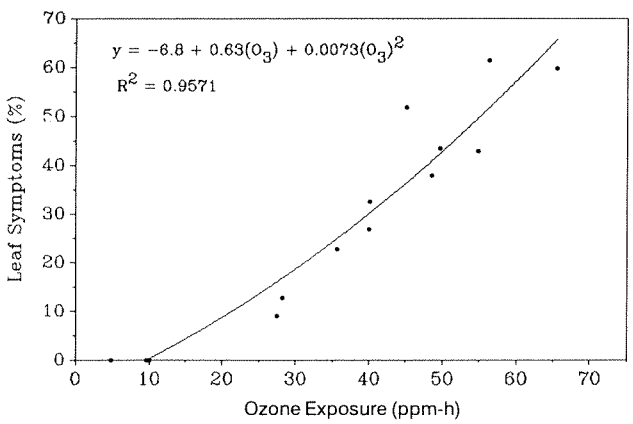
	Square wave[†] (52 ppm-h)				Episodic[‡]							
					70 ppm-h				92 ppm-h			
	Retained leaf	Stem	Root	Total	Retained leaf	Stem	Root	Total	Retained leaf	Stem	Root	Total
Clone 216	−33	−21	−35	−30	−16	−27	−22	−22	−26	−17	−34	−28
Clone 259	−32	−30	−53	−39	−22	−25	−27	−24	−36	−39	−55	−45
Clone 271	−18	−15	−34	−23	10	−9	−5	−2	−14	−8	−27	−19
All clones	−28	−22	−41	−30	−15	−20	−18	−16	−25	−21	−39	−31
Seedlings					−15	−20	−14	−14	−24	−19	−34	−26

Note: Values are the percent decrease as compared with charcoal-filtered air. The values for square wave are the means of 10 plants per clone. The values for the episodic exposures are the mean of 15 plants per clone. Decreases in biomass are based on the dry weight of the charcoal-filtered control plants.

[†]Consisted of 0.1 ppm ozone for 6 h/day, 4 days/week, for 12 weeks of the fumigation season.

[‡]The episodic 70 ppm-h and 92 ppm-h treatments were from the 1*x* and 2*x* treatments, respectively (Table 1).

Fig. 3. Leaf symptom (expressed as the percentage of the total leaves on plant) response to different total seasonal ozone exposures for plants of four *Populus tremuloides* clones. Potted, first-year plants were grown in open-top chambers and leaf injury was estimated late in the 1990 growing season. Each data point represents the average of 20 plants in the ozone exposure chamber. Symptomatic leaves were considered to be those showing typical ozone symptoms, including black bifacial necrosis, chlorosis, or upper surface stipple.



The early decreases in stem diameter and root biomass found in our study could have significant effects in subsequent years. However, our second-season reflush experiment suggested that even the most severely injured plants from the previous season had a strong capacity to recover when grown in low O_3 in the subsequent year. This conclusion is demonstrated by the fact that no significant differences occurred after reflush in bud-break phenology or amount of new stem and leaves produced among plants grown in any of the O_3 treatments. However, long-term studies are needed to examine the cumulative effects of O_3 on *P. tremuloides* over several growing seasons.

Fig. 4. Stem and foliage biomass (*a*) and root biomass (*b*) for *Populus tremuloides* clones. Potted, first-year plants were grown in open-top chambers at various ozone exposures for one season (1990) and then reflushed in clean air for 1 month in the subsequent year. The data are the average of 4 clones, 15 plants per clone, for a total of 60 plants each.

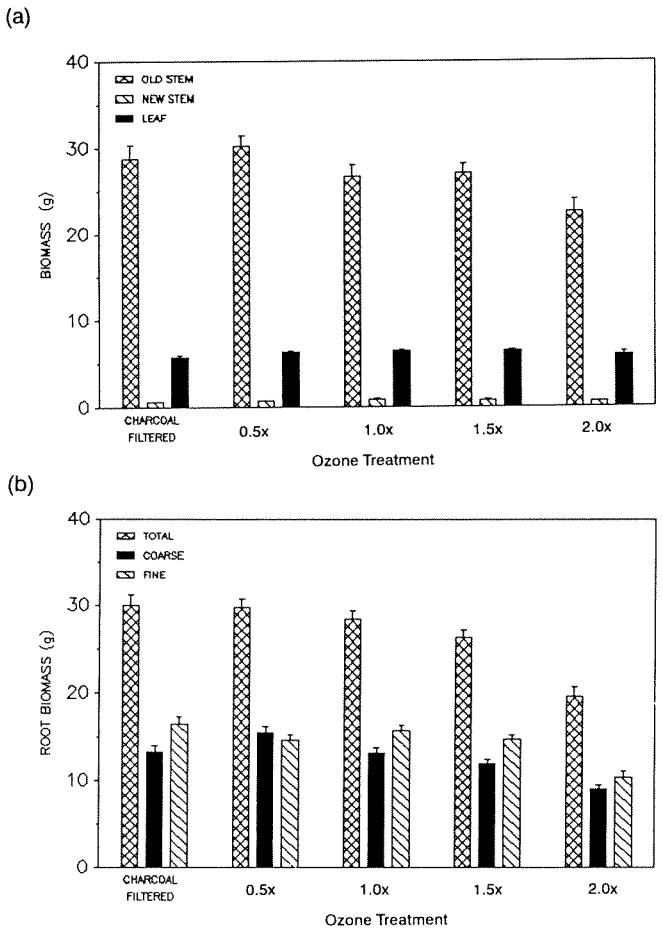


Table 9. Summary of multivariate analysis (probabilities) of *Populus tremuloides* seedlings exposed to ozone in open-top chambers and as potted first-year plants.

Effect[†]	Height	Diameter	Leaf abscission	Retained leaf biomass	Stem biomass	Root biomass	Total biomass
Tmt.	0.094	0.206	0.378[‡]	0.315[§]	0.438[§]	0.089[§]	0.191[§]
Time	0.599	0.456	0.123	na	na	na	na
Tmt.×time	0.178	0.247	0.324	na	na	na	na

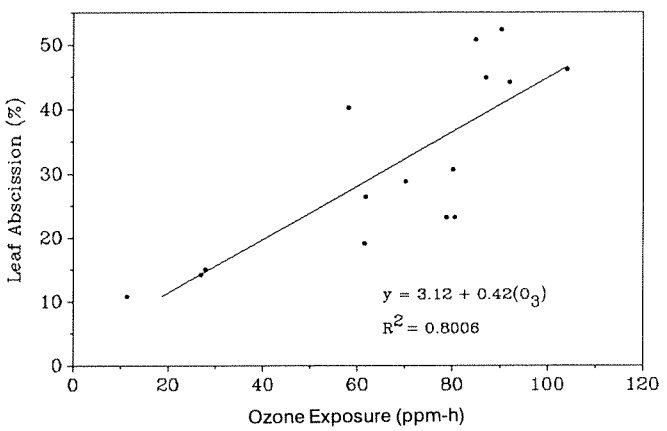
Note: na, not applicable, as no repeated measurements were taken.

[†]Tmt., ozone treatment; time, repeated measurements over the growing season.

[‡]Significance levels for treatment effects and the linear contrast on leaf abscission at the end of the growing season are 0.077 and 0.011, respectively.

[§]Significance levels for linear orthogonal polynomial contrast for leaf, stem, root, and total biomasses are 0.049, 0.151, 0.016, and 0.034, respectively.

Fig. 5. Leaf abscission response to different total seasonal ozone exposures for *Populus tremuloides* seedlings. Potted, first-year plants were grown in open-top chambers, and height was recorded late in the 1991 growing season. Each data point represents the average of 16 plants in each ozone exposure chamber.



Our results were consistent from year to year even though environmental conditions, plant size, and types of propagules were considerably different between the two growing seasons. These results corroborate our previous studies (Berrang et al. 1986, 1989, 1991; Karnosky 1976, 1977; Karnosky et al. 1992; Wang et al. 1986) which all suggested that *Populus* is genetically highly variable in O_3 sensitivity. Some *P. tremuloides* genotypes we examined are among the most O_3-sensitive trees known. The range of O_3 sensitivity we have identified is ideal for establishing a set of aspen bioindicators for O_3.

Our previous studies of O_3 effects on *P. tremuloides* have all examined clonally propagated plants. Because this species is propagated in nature both vegetatively by sucker sprouts from roots and sexually by seedlings (Andrejeak and Barnes 1969; Barnes 1966), it was important to also examine seedling responses to O_3. Our results suggest that seedlings are also sensitive to O_3. Similar trends in leaf abscission, growth, and biomass production were found for both clones and seedlings. Not surprisingly, however, coefficients of variation were generally higher for seedling responses as compared with the clonal responses. The larger variation in seedling responses to O_3 is indicative of the large amount of genetic variation one encounters when using seedlings as compared with clones. Thus, for physiological studies or long-term studies where the total number of plants is necessarily limited, clonal populations are useful for decreasing variability among experimental plants. By choosing clones with a range of sensitivities, as we did in this study, the experimenter can still elicit a wide range of responses. In addition, because within-replicate variability is decreased by the use of clonally propagated material, treatment responses are more likely to be statistically separable.

Plants exposed to two different exposure patterns of equal exposure showed greater reductions in growth and biomass production response to the square-wave exposure, which had more hours of 100 ppb than the episodic exposure. Possible explanations for these responses are, first, the episodic O_3 treatments allowed plants time to recover following the scattered high exposures, whereas, the square-wave regime allowed recovery only after 4 consecutive days of exposures. Second, the initial injury caused by the episodic peak exposures may have limited the subsequent injury. Third, the modified O_3 profile we used did not always provide high O_3 exposures at the time when plants were most photosynthetically active. Occasionally, high O_3 episodes also occurred in late afternoon or early evening, when gas exchange is generally decreased. While we cannot be sure if these possible explanations were the cause of this increased response in the square wave, the results have implications for future studies. While square-wave studies are useful for physiological and molecular studies where repeatable treatments are necessary, the more realistic episodic exposures should be preferred for growth studies.

A number of exposure-dynamic factors, including concentration, temporal pattern, predisposition, and respite times, as well as phenological stage of plant development, have been shown to influence the impact of O_3 on plant yield or biomass response (U.S. Environmental Protection Agency 1986, 1992, 1994). Because the total exposure index weighs all concentrations equally, the total exposure is inadequate for characterizing plant exposure to O_3

Table 10. Biomass (g) of potted, first-year *Populus tremuloides* seedlings exposed to different ozone treatments in open-top chambers in the summer of 1991 at Alberta, Mich.

Treatment[†]	Retained leaf	Stem	Root	Total
CF	6.0±0.6	5.9±0.7	9.7±0.9	22.0±2.1
0.5*x*	4.6±0.6	4.2±0.6	6.8±0.9	15.9±1.9
1.0*x*	4.8±0.6	4.4±0.7	8.1±1.0	18.1±2.1
1.5*x*	4.4±0.6	4.6±0.7	6.1±0.9*	15.6±2.1
2.0*x*	4.2±0.6	4.6±0.7	6.4±1.0	15.4±2.1
OP	4.0±0.6	3.6±0.7	8.6±1.1	16.5±2.3

Note: Values are means ± SE. Each value shown is the mean of 48 plants per treatment. Each clonal mean is based on 15 plants and is adjusted for initial volume.

[†]CF, charcoal-filtered air; *x*, ambient ozone level; OP, open-air plot data (not included in statistical analyses).

*Significantly lower than the charcoal-filtered treatment at the 0.05 level, as determined by Dunnett's test.

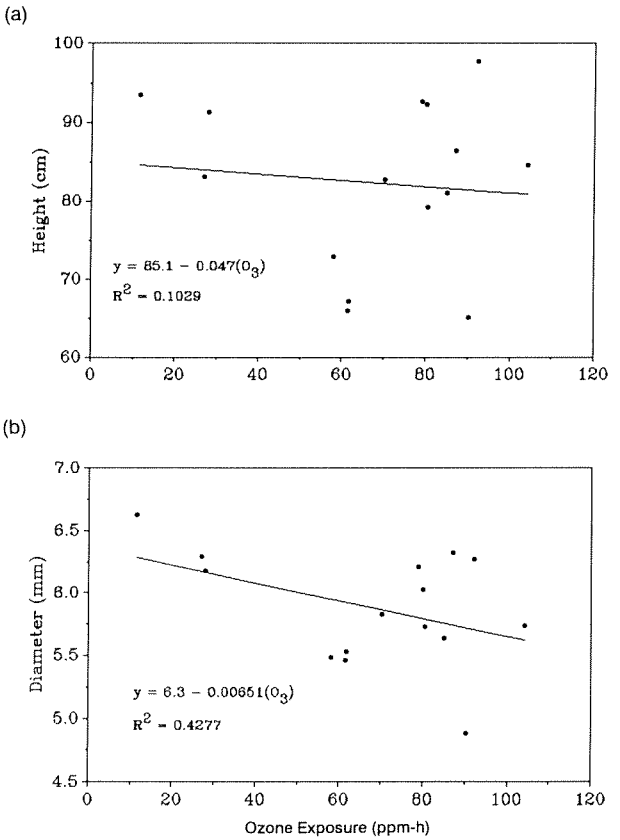
Table 11. Coefficients of variation (%) for *Populus tremuloides* clones and seedlings fumigated side by side in open-top chambers with episodic ozone exposures over the 1991 growing season.

	Variable				
Source	Height	Diameter	Retained leaf	Stem	Root
Clones[†]	16	12	34	36	36
Seedlings[‡]	24	20	43	51	48

[†]The values shown are the computed coefficients for a combined data set of 3 clones, 9 exposure chambers, and 5 plants per clone, for a total of 135 plants.

[‡]The values shown are the computed coefficients for a combined data set of all exposures and 16 plants per exposure chamber, for a total of 240 plants.

Fig. 6. Height (*a*) and stem diameter (*b*) responses to different total seasonal ozone exposures for *Populus tremuloides* seedlings. Potted, first-year plants were grown in open-top chambers, and height was determined late in the 1991 growing season. Each data point represents the average of 16 plants in each of the ozone exposure chambers.



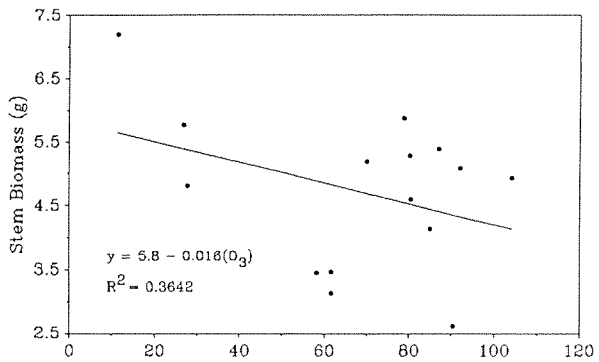
in relation to plant response (Lefohn et al. 1989). In one of the few comparable studies, Hogsett and Tingey (1990) found that growth reductions in both ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) and *P. tremuloides* were greatest in the episodic exposure pattern that had the largest peak concentrations of the three tested patterns, despite having equal total exposure.

The *P. tremuloides* growth responses we found in this study are sensitive to seasonal O_3 exposures similar to those occurring in much of our country. However, we know that one cannot directly extrapolate from an open-top chamber study to forest responses. Because we fumigated potted plants, and plant growth may be affected by the pot, the artificial soil mix, the fertilizer, and the watering regimes, there is a need for research on the effects of O_3 on field-grown *P. tremuloides* that are not subjected to artificial soil and pot conditions. Height growth and biomass of the chambered 1*x* plants were considerably greater than those in the open-air plots even though the O_3 exposures were similar, suggesting that a chamber effect was occurring. While open-top chambers are closer to forest conditions than either a greenhouse or growth chamber, at this time we cannot rule out the possibility that the chambers influenced O_3 responses of *P. tremuloides*. There is a definite need to conduct long-term studies to examine how *P. tremuloides* trees respond to multiple-season O_3 exposures, as our results cannot be extrapolated to several years of growth or to mature trees. While open-top chambers are closer to forest conditions than either a greenhouse or growth chamber, at this time we cannot rule out the possibility that the chambers influenced O_3 responses of *P. tremuloides*.

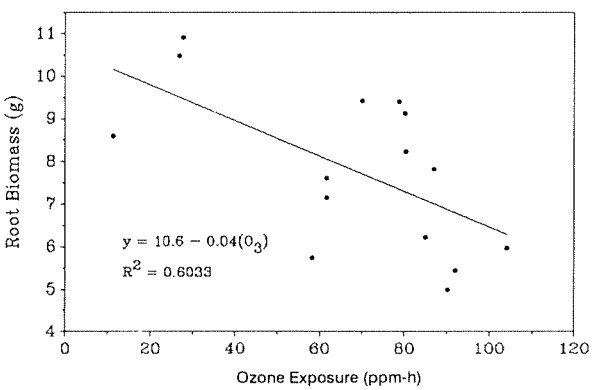
In summary, we have demonstrated that the clonal *P. tremuloides* material we have assembled has a wide range of responses to O_3. Therefore, it is excellent material for physiological and genetic mechanism studies of O_3. Several studies, designed to understand the fundamental mechanisms of O_3 response in trees employing these clones, are underway (Coleman et al. 1995*a*, 1955*b*; Gagnon et al. 1992; Li et al. 1991).

Fig. 7. Stem (*a*) and root (*b*) biomass responses to different total seasonal ozone exposures for *Populus tremuloides* seedlings. Potted, first-year plants were grown in open-top chambers and biomass was determined late in the 1991 growing season. Each data point represents the average of 16 plants in each of the ozone exposure chambers.

(a)



(b)



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